

Cardiovascular effects of intravenous ghrelin infusion in healthy young men

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Vestergaard ET, Andersen NH, Hansen TK, Rasmussen LM, Møller N, Sørensen KE, Sloth E, Jørgensen JO. Cardiovascular effects of intravenous ghrelin infusion in healthy young men. *Am J Physiol Heart Circ Physiol* 293: H3020–H3026, 2007. First published September 14, 2007; doi:10.1152/ajpheart.00496.2007.—Ghrelin infusion improves cardiac function in patients suffering from cardiac failure, and bolus administration of ghrelin increases cardiac output in healthy subjects. The cardiovascular effects of more continuous intravenous ghrelin exposure remain to be studied. We therefore studied the cardiovascular effects of a constant infusion of human ghrelin at a rate of 5 pmol/kg per minute for 180 min. Fifteen healthy, young (aged 23.2 ± 0.5 yr), normal-weight (23.0 ± 0.4 kg/m²) men volunteered in a randomized double-blind, placebo-controlled crossover study. With the subjects remaining fasting, peak myocardial systolic velocity S' , tissue tracking TT, left ventricular ejection fraction EF, and endothelium-dependent flow-mediated vasodilatation were measured. Ghrelin infusion increased S' 9% ($P = 0.002$) and TT 10% ($P < 0.001$), whereas EF, resting blood flow velocity, and endothelium-dependent flow-mediated vasodilatation did not change ($P = 0.13$). This was associated with a peak in serum growth hormone after 60 min of infusion (37.77 ± 5.27 ng/ml, $P < 0.001$), a doubling of free fatty acid levels ($P = 0.001$), and a 1.6-fold increase in cortisol levels ($P < 0.05$), whereas glucose and catecholamine levels were constant. In conclusion, supraphysiological levels of ghrelin stimulate left ventricular function in terms of S' and TT in healthy young normal-weight men without changing resting blood flow velocity and endothelium-dependent flow-mediated vasodilatation. The effects did not translate into detectable increments in EF.

left ventricular function; tissue Doppler imaging; tissue tracking; endothelium-dependent flow-mediated vasodilatation

GHRELIN IS THE ENDOGENOUS ligand for the growth hormone (GH) secretagogue receptor type 1a (GHS-R1a) (27).

Administration of ghrelin is associated with a distinct stimulation of GH secretion, but ghrelin also induces the release of adrenocorticotrophic hormone (ACTH) and prolactin (41).

The GHS-R1a and its mRNA are not only restricted to the hypothalamus and the pituitary gland but are also located in various human tissues including the myocardium (18, 21, 23, 33, 38). Ghrelin ($[^{125}\text{I-His}^9]$ -ghrelin) demonstrates a saturable, reversible, and high-affinity binding to human cardiovascular tissue (23), and the highest binding activity of $[^{125}\text{I}]\text{Tyr-Ala-hexarelin}$ (a synthetic GHS-R1a ligand) has been observed in the myocardium (38).

In vitro, ghrelin protects the cardiomyocyte cell line H9c2 against doxorubicin-induced apoptosis (5), and, in vivo, ghre-

lin protects rat myocardium against isoproterenol-induced myocardial injury (12) and reduces infarct size following ischemia (17).

In healthy subjects, exogenous ghrelin injected as an intravenous bolus acutely increases cardiac output (33). In a placebo-controlled, randomized noncrossover study comprising 12 patients with chronic heart failure, a 60-min constant high-dose infusion of ghrelin (more than 5 times the dose used in the present study) improved cardiac and stroke volume index concomitantly with a significant increase of epinephrine levels and a decrease of cardiac afterload (34).

Besides putative direct cardiac effects, ghrelin may exert indirect effects via an increase in GH (14, 33–35) and catecholamine levels (33, 34) and a decrease in cardiac afterload (33, 34). However, the cardiovascular effects have been investigated in subjects with high ghrelin levels only (more than 12 times higher than peak fasting levels) (14, 34).

Reports on the localization of the receptor for ghrelin (18, 21, 23, 33, 38) and on the cardiovascular effects of ghrelin (5, 12, 14, 17, 33–35) substantiate the importance to evaluate in more details potential cardiovascular therapeutic applications of exogenous ghrelin.

We hypothesize that ghrelin improves cardiovascular functions independently of vasodilatation. In this randomized, double-blind, and placebo-controlled crossover study, we investigated the effects of more physiological increments in ghrelin levels on left ventricular peak myocardial systolic velocity, distance of motion along the Doppler axis (tissue tracking), and endothelium-dependent vasodilatation in humans.

MATERIALS AND METHODS

Study Subjects

Fifteen healthy men, aged 23.2 ± 0.5 yr with a body mass index of 23.0 ± 0.4 kg/m², volunteered for this study. The participants had no history or present symptoms of cardiovascular disease, vasculitis, or coagulopathy, and none were smokers, abusers of alcohol, or taking any type of medication including aspirin. They all had a normal physical examination including heart rate (61 ± 2 per min) and blood pressure (systolic 125 ± 2 , diastolic 79 ± 2 mmHg). Fasting blood glucose, triglycerides, and cholesterol levels, hematological, renal, and hepatic functions assessed by biochemical screening were also normal in all participants.

The study was conducted in accordance with the Helsinki Declaration and all subjects gave their oral and written, informed consent to participate. The study protocol was approved by the local Ethics

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Committee of Aarhus County, the Danish Medicines Agency and the Good Clinical Practice (GCP) Unit of Aarhus University Hospital. According to the International Committee of Medical Journal Editors, the protocol was registered (Clinicaltrials.gov ID NCT00116025) before the onset of enrollment.

Data focusing on pharmacokinetics from this study have previously been published (45).

Preparation of Synthetic Ghrelin

Synthetic human acylated ghrelin (NeoMPS, Strasbourg, France) was dissolved in isotonic saline and sterilized by double passage through a 0.8- and 0.2- μ m-pore-size filter (Super Acrodisc, Gelman Sciences, Ann Arbor, MI). The preparation was made by the local hospital pharmacy.

Protocol

In a randomized double-blind and placebo-controlled crossover design we used a 3-h constant infusion of human ghrelin (infusion period $t = 0$ to 180 min) of 5 pmol/kg per minute to investigate effects on systolic myocardial velocities, tissue tracking (TT), left ventricular ejection fraction, and flow-mediated vasodilatation of the radial artery (FMD-R). Seven subjects were randomized to receive ghrelin on the first and saline on the next study day. The remaining eight subjects were randomized to the opposite treatment sequence. The study days were separated by a minimum of 3 wk. Fasted subjects had two intravenous cannulae inserted in the cubital regions: one for infusion, and, in the contralateral arm, one for blood sampling. The intravenous line was kept patent during both ghrelin and placebo administration by infusion of an isovolumetric load of isotonic saline at a rate of 1.69 ml/kg body wt per hour. The subjects were studied in the supine position from 8:00 AM during quiet, thermoneutral conditions. Echocardiography was performed before infusion period ($t = -15$), after 60 min ($t = 60$), and after 165 min from onset of infusion ($t = 165$). Flow-mediated vasodilatation was measured on the right-hand side before the infusion period ($t = -30$) and after 150 min of infusion ($t = 150$). The subjects fasted during the trial.

Echocardiographic Study

Echocardiography. All subjects underwent an echocardiographic examination performed by the same observer with all post hoc off-line analyses being performed blinded to the randomization. Echocardiography was performed on a GE Vivid Seven (GE Healthcare, Horten, Norway) with a 2.5-MHz transducer, using second harmonic modalities for enhanced endocardial border detection (24).

Tissue Doppler imaging. Tissue Doppler imaging was obtained from the four- and two-chamber apical views and from the apical long-axis view during end-expiration apnea.

The left ventricle was divided into 12 segments (septal, lateral, anterior, inferoseptal, antero-septal, and posterior segments at both the basal and middle level) and the peak myocardial systolic velocities and distance of motion for each segment were assessed. The peak systolic velocity and distance of motion were assessed as the mean of the values obtained from three consecutive heart cycles from each of the 12 myocardial segments. The averages of the 12 segments are presented and denoted S' for peak systolic velocity and TT for the distance of motion. The tissue Doppler technique in the present study allowed processing of simultaneous S' and TT from different myocardial segments in the same cine-loop.

TT displays the integral of tissue velocity during systole, which equals the distance of motion along the Doppler axis (3, 37). TT requires simultaneous ECG registration to define the beginning and the end of the systole. Seven color bands indicate different distances of motion with a stepwise increase in the distance of motion. The range of distance of motion displayed by the seven color bands can be altered depending on the left ventricular function, to stretch the color

bands between the apex and the mitral annular level. Analyzing the left ventricle in apical views, the lowest distance of motion is at the apex and the greatest at the mitral annulus.

Color noise reduction was adjusted and the tissue Doppler scanning frame rates were kept above 160 frames per second in all cases (1). Loading conditions were kept similar during ghrelin infusion and placebo treatment (4).

Ejection fraction. Left ventricular ejection fraction was estimated using Simpson's modified biplane method, based on three measurements. Endocardial border detection was enhanced by use of second harmonic imaging (25, 29).

Vascular Study

Changes in the proximal right-hand-side radial artery diameter in response to reactive hyperemia were measured right below the intravenous cannulae in the cubital region with high-resolution ultrasound (Acuson Sequoia C256, Siemens, Mountain View, CA, with an 8-MHz linear array vascular transducer). Endothelium-dependent flow-mediated vasodilatation of the radial artery was carried out as previously recommended (13). After ~ 30 min of rest, the intima-media boundaries were identified manually and the baseline diameter of the radial artery and the blood flow were recorded. Arterial blood flow was estimated by calculating the velocity-time integral (VTI) corresponding to the area under the Doppler curve. The transducer was held in place by a stereotactic clamp, and a blood pressure cuff was placed proximal to the cubital fossa and inflated to ~ 280 mmHg. After 5 min, the cuff was quickly deflated, whereafter a midartery pulsed Doppler signal was obtained and artery diameter measurements were taken for 2 consecutive min. A second measurement of flow-mediated vasodilatation was performed just prior to termination of the infusion period. Finally, endothelium-independent nitroglycerine-mediated vasodilatation was performed 4 min after sublingual administration of nitroglycerine spray (0.4 mg).

All images were digitized online and stored for later off-line analyses. Straight segments of the artery (more than 10 mm if possible) were chosen for analysis and the artery diameter was measured at the same time in the cardiac cycle (with the R wave on the ECG). All procedures were done, blinded to the randomization sequence, by the same experienced observer. The image analysis and measurement of the vasodilator response were performed in triplicate.

Biochemical Measurements

Serum ghrelin (total levels) were measured in duplicate by an in-house assay as described previously (16). The assay measures immunoreactive levels of ghrelin using 125 I-labeled bioactive ghrelin tracer and rabbit polyclonal antibodies raised against octanoylated human ghrelin. The assay recognizes the COOH-terminal of ghrelin and as such determines acylated as well as des-acylated ghrelin. The intra-assay coefficient of variation averaged 2.8% and samples from each individual were analyzed in one assay. A commercial double monoclonal immunofluorometric assay (DELFA, Perkin Elmer, Wallac, Turku, Finland) was used to measure serum GH (s-GH), cortisol (s-cortisol), and insulin (s-insulin). Plasma glucose (p-glucose) levels were measured in duplicate on a glucose analyzer (Beckman Instruments, Palo Alto, CA). Serum free fatty acids (s-FFA) were determined by use of a commercial kit (Wako Chemicals, Neuss, Germany). Plasma catecholamines were measured by liquid chromatography (15). Serum soluble intercellular cell adhesion molecule-1 (s-ICAM-1) and vascular cell adhesion molecule-1 (s-VCAM-1) were measured by commercially available ELISA kits, as described by the manufacturer (R&D Systems, Minneapolis, MN, catalogue nos. BBE1B and DY809). Serum concentration of osteoprotegerin (OPG) was measured in duplicate by a sandwich ELISA method (R&D Systems, Minneapolis, MN, catalog no. DY805).

Statistical Analysis

Results are expressed as means \pm SE. S' , TT, and heart rate were analyzed by two-way ANOVA. The treatment \times time interaction was considered the term of interest. The Bonferroni correction was used to account for multiple comparisons. To adjust for the considerable (9) pure biological, within-subject variability during the course of 1 day in FMD-R, resting blood flow, peak hyperemic flow, and arterial diameter, we compared the differences in FMD-R and the vascular parameters during ghrelin treatment with differences observed during placebo treatment. The trapezoidal rule was used to estimate area under the concentration curve (AUC) levels for the hormones and metabolites. FMD-R, hormones, and metabolites were examined by Student's two-tailed paired t -test. A P value <0.05 was chosen as level of significance. All analyses were performed with SPSS version 14.0 for Windows.

RESULTS

Echocardiographic Study

The mean peak systolic velocities were similar at baseline in the saline and ghrelin groups (Table 1). S' increased by $9.1 \pm 2.6\%$ during the first 60 min of ghrelin infusion, $P = 0.007$, and the increase persisted throughout the study. S' was constant during saline infusion, $P = 0.17$, Fig. 1. There was a significant effect of ghrelin infusion on S' , P (time \times treatment) = 0.002.

The heart rates were comparable between groups and stable over time, P (time \times treatment) = 0.69, Table 1.

Tissue Tracking

TT, reflecting the global longitudinal systolic contraction amplitude of the left ventricle, increased significantly by ghrelin infusion with 9.6 ± 3.1 and $11.2 \pm 3.6\%$ after 60, $P = 0.013$ and 165 min, $P = 0.010$, respectively. TT was unaffected by saline infusion, $P = 0.19$, Fig. 2. Overall, we found a significant effect of ghrelin infusion on TT, P (time \times treatment) < 0.001 .

Ejection Fraction

The three repeated measures of left ventricular ejection fraction during ghrelin and saline infusion were similar, P (time \times treatment) = 0.92, Table 2.

Table 1. Effects of ghrelin and saline (placebo) on systolic velocities and tissue tracking

Time, min	Treatment	
	Ghrelin	Placebo
Systolic velocity, cm/s		
–15	6.8 ± 0.3	7.3 ± 0.3
60	7.5 ± 0.3	7.0 ± 0.3
165	7.5 ± 0.4	7.1 ± 0.4
Tissue tracking, mm		
–15	11.5 ± 0.4	12.1 ± 0.3
60	12.5 ± 0.4	11.8 ± 0.3
165	12.7 ± 0.5	11.9 ± 0.3
Heart rate, per min		
–15	58.1 ± 1.8	53.2 ± 2.1
60	54.5 ± 1.2	51.4 ± 1.9
165	54.4 ± 1.1	51.1 ± 1.8

Values are means \pm SE.

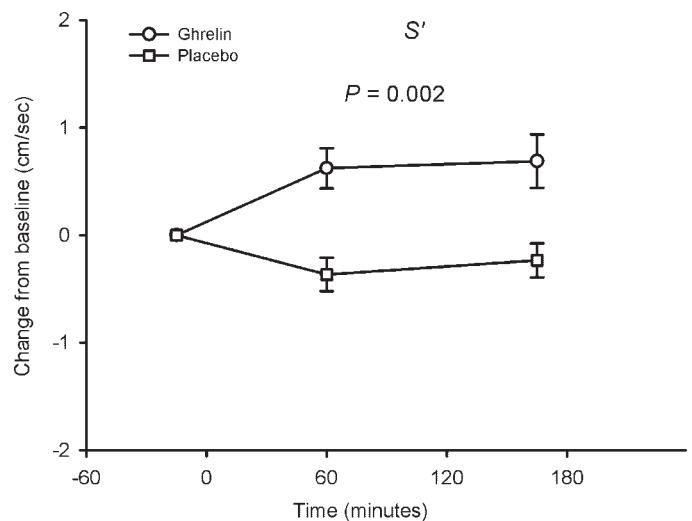


Fig. 1. Systolic velocities (S') in the ghrelin (○) and placebo group (□) at different time points. Velocities are shown as changes from baseline values. Printed P value refers to the interaction of time and treatment of ghrelin on S' .

Vascular Study

VTI, peak hyperemic flow, diameter of the radial artery, and the FMD-R at baseline were similar on each of the 2 study days (see Table 3 for details).

When the delta values are compared from time $t = -30$ to 150 min of ghrelin- and saline-infusion, neither ghrelin nor saline had any effect on the vascular measurements including FMD-R (Table 4).

Ghrelin infusion showed a trend to decrease VTI ($t = -30$ min vs. $t = 150$ min: $P = 0.08$), whereas ghrelin infusion had no effects on resting diameter of the radial artery, peak hyperemic blood flow, or FMD-R, P all > 0.05 .

Endothelium-independent (nitroglycerine-mediated) vasodilatation was also similar during ghrelin- ($12.1 \pm 1.5\%$) and saline-infusion ($15.2 \pm 2.1\%$), $P = 0.13$.

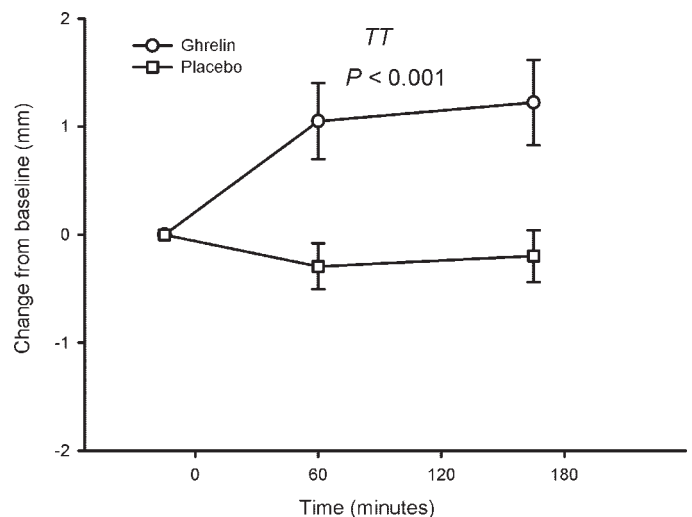


Fig. 2. Tissue tracking (TT) in the ghrelin (○) and placebo (□) groups at different time points. Distances of motion along the Doppler axes are shown as changes from baseline values. Printed P value refers to the interaction of time and treatment of ghrelin on TT.

Table 2. Effects of ghrelin and saline on left ventricular ejection fraction

Time, min	Treatment	
	Ghrelin	Placebo
-15	55.1±0.7	55.3±0.6
60	56.6±1.0	56.9±0.7
165	57.1±0.8	57.0±0.8

Values are means ± SE. Ejection fraction is in percentage (%).

Hormones and Metabolites

The effects of ghrelin infusion on hormones and metabolites are reported in Table 5.

Baseline serum ghrelin levels were $0.72 \pm 0.06 \mu\text{g/l}$ and increased ~5.0- and 6.7-fold above baseline levels after 60 and 165 min, respectively.

Serum levels of GH and cortisol both peaked after 60 min, both $P < 0.05$, thereafter decreasing. Serum cortisol levels were similar at $t = 165$ min, $P = 0.054$, whereas the level of GH at this time point remained elevated by ghrelin infusion, $P < 0.05$. s-FFA levels reached a significantly higher level during ghrelin infusion at $t = 165$ min compared with placebo infusion, whereas glucose levels were unaltered in this study, $P = 0.90$ and $P = 0.30$ at $t = 60$ and $t = 165$ min, respectively. Serum insulin levels were also unaffected by the infusion of ghrelin at $t = 60$ min, $P = 0.62$, but increased at $t = 165$ min, $P < 0.05$. Catecholamine levels were similar on the two occasions, all P values > 0.05 for both epinephrine and norepinephrine at $t = 60$ and 165 min, respectively.

Markers of Endothelial Activation and Vascular Matrix Changes

Serum levels of s-ICAM-1, s-VCAM-1, and OPG were similar at baseline (ghrelin vs. placebo): $189.0 \pm 6.6 \mu\text{g/l}$ vs. $198.1 \pm 9.2 \mu\text{g/l}$, $P = 0.054$, $291.0 \pm 16.2 \mu\text{g/l}$ vs. $296.8 \pm 18.3 \mu\text{g/l}$, $P = 0.41$, and $1.2 \pm 0.1 \mu\text{g/l}$ vs. $1.3 \pm 0.2 \mu\text{g/l}$, $P = 0.30$, respectively. Ghrelin infusion had no effect on the levels of either of these analytes (ghrelin vs. saline): AUC for s-ICAM-1 $67.2 \pm 2.5 \text{ mg/l} \times \text{min}$ vs. $70.3 \pm 3.8 \text{ mg/l} \times \text{min}$, $P = 0.12$, AUC for s-VCAM-1 $104.9 \pm 6.0 \text{ mg/l} \times \text{min}$ vs. $104.2 \pm 6.3 \text{ mg/l} \times \text{min}$, $P = 0.87$, and AUC for OPG $414.8 \pm 54.8 \mu\text{g/l} \times \text{min}$ vs. $438.3 \pm 53.9 \mu\text{g/l} \times \text{min}$, $P = 0.63$.

DISCUSSION

The study shows that a constant ghrelin infusion stimulates load-independent indexes of myocardial function such as the left ventricular peak systolic velocity and contraction ampli-

Table 3. Baseline characteristics of resting VTI, peak hyperemic flow, diameter of the radial artery, and FMD-R at $t = -30$ min on each of the 2 study days

Variable	Ghrelin	Placebo	P
Resting VTI, cm	3.4±0.6	3.5±0.6	0.91
Peak hyperemic flow, %	1,121±141	1,073±137	0.71
Resting diameter, mm	2.60±0.09	2.57±0.10	0.67
FMD-R, %	7.4±1.2	9.2±1.4	0.22

Values are means ± SE. VTI, velocity-time integral; FMD-R, flow-mediated vasodilatation of the radial artery.

Table 4. Delta values of resting VTI, peak hyperemic flow, diameter of the radial artery, and FMD-R from time $t = -30$ to 150 min of ghrelin- and saline-infusion, respectively

Variable	Ghrelin	Placebo	P
ΔResting VTI, cm	-0.5±0.3	-0.2±0.3	0.22
ΔPeak hyperemic flow, %	153±108	37±58	0.21
ΔResting diameter, mm	0.03±0.03	0.00±0.03	0.51
ΔFMD-R, %	-0.1±0.7	1.8±0.9	0.13

Values are means ± SE.

tude without affecting resting blood flow in the radial artery or endothelium-dependent flow-mediated vasodilatation. There was no effect on conventional ejection fraction.

Acute inotropic effects have previously been shown after an intravenous bolus administration of a pharmacological dose, resulting in a 60-fold increase in systemic ghrelin levels (33), and similar effects have been found in patients with congestive heart failure (34, 35).

Besides putative direct cardiac effects, ghrelin may exert indirect effects through an increase in GH (14, 33–35) or catecholamine levels (33, 34), or via a decrease in cardiac afterload (33, 34).

Ghrelin has been shown to improve endothelial dysfunction by increasing endothelial nitric oxide (NO) release in GH-deficient rats (40), as well as in patients with the metabolic syndrome (42). Moreover, in healthy humans, intra-arterial ghrelin showed GH- and NO-independent vasodilatory properties (36). Such endothelial-independent vasodilatory effects of ghrelin may be mediated by physiological antagonism of endothelin-1 (50).

Table 5. Circulating concentrations of ghrelin, growth hormone, cortisol, insulin, epinephrine, norepinephrine, glucose and free fatty acids in 15 healthy volunteers during the infusion of ghrelin and placebo. * refers to $P < 0.05$ by post hoc comparison between ghrelin and placebo

	-15 min	60 min	165 min
Ghrelin, $\mu\text{g/l}$			
Ghrelin	0.72±0.06	3.93±0.24*	5.32±0.34*
Placebo	0.75±0.07	0.78±0.07	0.79±0.07
GH, ng/ml			
Ghrelin	0.36±0.25	37.77±5.27*	8.52±1.81*
Placebo	0.74±0.47	1.59±0.64	0.94±0.26
Cortisol, nmol/l			
Ghrelin	277.1±24.6	338.3±31.8*	290.3±26.9
Placebo	312.9±27.3	216.9±23.2	231.9±24.7
Insulin, pmol/l			
Ghrelin	25.1±1.9	19.9±2.1	25.0±2.6*
Placebo	25.9±3.6	21.2±2.6	18.8±2.2
Epinephrine, pg/ml			
Ghrelin	46.8±4.8	45.9±6.5	44.7±5.8
Placebo	38.5±4.5	37.3±3.9	41.4±8.3
Norepinephrine, pg/ml			
Ghrelin	207.4±19.5	184.4±22.5	205.2±20.1
Placebo	205.2±28.4	197.3±18.1	220.8±26.6
Glucose, mmol/l			
Ghrelin	4.9±0.1	5.0±0.1	5.3±0.1
Placebo	5.1±0.1	5.0±0.1	5.2±0.1
FFA, mmol/l			
Ghrelin	0.39±0.05	0.59±0.07	1.04±0.09*
Placebo	0.34±0.05	0.44±0.07	0.51±0.07

Values are means ± SE. * $P < 0.05$ vs. placebo.

In the present study s-GH increased rapidly in response to ghrelin infusion, reaching the peak level after 60 min of infusion and decreasing hereafter. The acute cardiovascular effects (within hours) of GH are, if any, disputable (47, 51). In normal cardiac myocytes GH neither increased intracellular Ca^{2+} nor increased contractility (26). There is more evidence in support of GH-independent cardiovascular effects of ghrelin; in rodent studies, hexarelin and ghrelin, both independently of GH, attenuated myocardial injury and left ventricular dysfunction after ischemia-reperfusion injury (11, 30), and in GH-deficient patients an intravenous bolus of hexarelin improved GH-independent left ventricular ejection fraction (7, 8).

G protein-coupled receptor (GPCR) activation initiates cardiomyocyte signaling, leading to adaptive cardiac function. Included among the cardiac GPCR is the most widespread ghrelin receptor GHS-R1a (23, 38). GHS-R1a is considered to signal via $\text{G}\alpha_q$ and thus activates phospholipase C, which results in increased inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) levels. IP_3 triggers the mobilization of free calcium (Ca^{2+}) from the endoplasmic reticulum and DAG activates PKC. PKC inhibits K^+ channels, and the following depolarization causes an increased Ca^{2+} current (10). In rodent models ghrelin partitioned whole body metabolism toward the utilization of glucose rather than fat (44) and decreased the hepatic mitochondrial FFA uptake enzyme carnitine palmitoyl transferase-1 (6). Together, those events may explain the positive inotropy observed in healthy humans, but specific cardiomyocyte ghrelin-signaling has not been fully elucidated and the above described mechanisms remain to be documented in myocardial tissue. Available in vitro data report that ghrelin increases protein kinase B and ERK 1 and 2 phosphorylation in H9c2 cardiomyocytes and improves cardiomyocyte survival when coincubated with doxorubicin (5). In rodents, ghrelin administration results in cardiac AMP-activated protein kinase activity (28), which mediates insulin-independent glucose uptake, thereby influencing cell metabolism. Our present study conducted in healthy volunteers describes clinical effects of ghrelin on the left ventricle function only. Future studies should be performed to investigate the cardiac metabolic effects of ghrelin and in a future series of studies we aim to apply PET technique to investigate these issues.

Decreased cardiac afterload by peripheral vasodilatation can explain an increase in cardiac function. In this study, however, ghrelin exerted no effects on either heart rate, arterial diameter, resting blood flow velocity, or endothelium-dependent vasodilatation, when the radial artery was probed with a method that previously has proven reliable (20), and, if anything, ghrelin showed a tendency to decrease resting blood flow. We did not record systemic blood pressure, which is a limitation of the present study. The effect of ghrelin on blood pressure has, however, been investigated in other clinical trials. Enomoto et al. (14) injected three doses of ghrelin (1, 5, and 10 $\mu\text{g/kg}$) to humans, which resulted in 2-, 8-, and 12-fold increases of baseline ghrelin values and no effect on mean arterial pressure. In other trials, ghrelin levels were increased by 43- (34) and 61-fold increases (33), which resulted in a 9- and 12-mmHg reduction in mean arterial blood pressure, respectively. In comparison, the 6.7-fold increase of ghrelin compared with the placebo value in this study is in the range with no expected blood pressure effects. On the basis of existing literature we had anticipated an endothelial-dependent vasodilation after

ghrelin (36, 42). Endothelial function is, however, also regulated by metabolic cues. In particular, insulin resistance and elevated levels of FFA may impair endothelium-dependent vasodilatation (43, 46). We have recently observed that exogenous ghrelin induces acute insulin resistance in skeletal muscle in concomitance with elevated FFA levels (unpublished data). It is therefore plausible that ghrelin via both direct and indirect mechanisms may exert reciprocal effects on endothelial function. The roles of epinephrine, norepinephrine, and soluble adhesion molecules are probably minor in the present study, because the levels of all three hormones and markers of endothelial activation were similar during both placebo and ghrelin treatment. Indeed, insulin levels increased in the present study, but the increase was only recorded when the study was terminated.

OPG, a member of the tumor necrosis factor receptor superfamily, is expressed in arterial wall and increased in diabetic patients. In this study, the infusion of ghrelin translated into metabolic impairment in terms of elevated FFA levels, whereas OPG levels were constant. It is of significant interest whether ghrelin downregulates OPG in subjects expressing increased levels of FFA and insulin, but our infusion period may be too short to uncover augmented levels of OPG. Clearly, long-term studies are needed to clarify this matter.

We also observed a significant increase in the circulating levels of cortisol in response to ghrelin administration. The effect of cortisol per se on left ventricular function in vivo is largely unknown. A limited number of reports describe the role of glucose and FFA on left ventricular function in vivo; contractile function of the normal heart is not improved by an acute increase in glucose uptake (39), and FFAs have no effect on contractility in either chronically stunned, hibernating, or control myocardial regions (49).

Well-known pharmacological effects of ghrelin include increased systemic levels of GH, ACTH, cortisol, prolactin, and epinephrine (33, 41), whereas more physiological ghrelin increments (i.e., 2- to 3-fold elevations) usually have no significant effects on the levels of metabolites and hormones except GH (2, 31, 52). We hypothesized that ghrelin stimulates cardiovascular functions and the aim of this study was to investigate the sustained effects of more moderate increments in ghrelin levels compared with previous studies (14, 33–35). We used a ghrelin dose of 5 pmol/kg per minute, corresponding to approximately half-maximal GH stimulating dose (52). Since a previous study showed that 2 h of ghrelin infusion at 7.5 pmol/kg per minute did not influence glucose, insulin, IGF-I, cortisol, epinephrine, norepinephrine, or FFA levels, and the GH response was most distinct within the first 90 min, resolving thereafter (31), we were surprised to find an increase in cortisol and FFAs in our study.

The present study is distinct from earlier studies in several respects. First, earlier studies of the cardiac effects of ghrelin in humans have used either invasive methods (33, 34) or echocardiographically assessed left ventricular ejection fraction (14, 35), which are both dependable on pre- and afterload conditions. We used tissue Doppler imaging and TT techniques, which are sensitive noninvasive methods for assessing systolic tissue velocities and longitudinal displacement of the myocardium (3). In addition, both measures are more reliable indexes of the myocardial function rather than reflecting changes in pre- and afterload conditions (37, 48). This is of

significant interest because ghrelin administration has been shown to decrease afterload (33, 34). Second, earlier studies used very high supraphysiological or pharmacological ghrelin doses (14, 33, 34). Third, the majority of earlier studies used bolus administration of ghrelin (14, 22, 33, 35) instead of a continuous infusion. Fourth, some of the previous studies lacked a placebo group (22, 35). Finally, clinical studies of the vasoactive effects of ghrelin used intra-arterial administration (36, 42), whereas the present study reports the effects of elevated systemic ghrelin levels.

Compensatory mechanisms suppress ghrelin levels in obesity (19), and concomitant insulin resistance contributes to a further suppression (32). Although correlation does not imply causality, it is tempting to hypothesize that suppressed ghrelin levels may contribute to the cardiovascular morbidity in obese and insulin-resistant subjects. Conversely, weight loss in obese patients, which increases circulating ghrelin levels (19), could theoretically improve cardiac function. In a clinical setting, treatment with ghrelin or one of its analogs could become a future option for patients suffering from chronic heart failure and cachexia.

The present study for the first time describes that in a randomized, double-blind, placebo-controlled crossover study supraphysiological ghrelin levels persistently throughout the 3-h study period stimulate left ventricular function, assessed by a noninvasive technique, without changing endothelium-dependent vasodilatation. The effects did, however, not translate into detectable increments in ejection fraction, and a longer infusion period would be needed to substantiate the potential effect of ghrelin in chronic heart failure.

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